EXHIBIT 46

USER'S GUIDE TO BIO-PROBE™ LABELING SYSTEMS

Biotin-Labeling strategies from Enzo Biochem . . .

☑ . . . Nick Translation Kit

- For incorporation of biotinylated nucleotides into double-stranded DNA.
- The most widely applied procedure for labeling double-stranded DNA.
- Biotinylated DNA probes exhibit normal hybridization kinetics.

☑ . . . Terminal Labeling Kit

- Template-independent addition of biotinylated polynucleotides to 3′-OH termini.
- Applicable to labeling double-stranded DNA, single-stranded DNA, cDNA and long and short probes.
- Standard hybridization conditions are used.

☑ . . . *Bio-Bridge*™ Labeling System

- Indirect delivery of biotin label to DNA probes by a polynucleotide moiety which contains biotinylated residues and is capable of hybridizing to the probe DNA.
- Eliminates need for direct biotinylation of probe.
- Applicable to a wide spectrum of DNA probes.

☑ . . . rIbo *Bio-Probe*™ Kit

- For incorporating biotinylated nucleotides into RNA through transcription of cloned sequences.

ENZO

Biotin-Labeling Strategies from **Enz Bi h m:**

Enzo has developed labeling procedures for a wide variety of research needs. Each complete labeling kit contains all the necessary reagents and a detailed protocol for the preparation of biotinylated DNA or RNA probes.

Nick Translation Kit*

Biotin labeling of DNA by nick translation results in the incorporation of biotinylated nucleotide(s) into double-stranded DNA.

Nick translation (1) is based on the introduction of random scissions, or "nicks", by pancreatic DNase 1. *E. coli* DNA polymerase I then catalyzes the sequential addition of nucleotide residues to the 3'-hydroxyl terminus of a nick, with the simultaneous elimination of nucleotides from the 5'-phosphoryl terminus. There is no net DNA synthesis. As nucleotides are removed from the 5'-phosphoryl terminus and new ones added to the 3'-hydroxyl terminus, the nick is moved, or translated, linearly along the strand. In the presence of biotinylated nucleotide(s) pre-existing unmodified nucleotides in the DNA strand are replaced by biotinylated analogs.

Enzo's Nick Translation Kit follows established protocols, using Bio-11-dUTP, a TTP analog, as the biotinylated nucleotide. Because nick translation is a template-dependent reaction, the number of biotinylated nucleotides incorporated is determined by the A-T composition of the DNA. With this procedure 20-60% of the thymidine residues in the probe are replaced with Bio-11-dUMP.

Nick translation is recommended for biotinylating double-stranded DNA greater than I kb. For shorter probes, care must be taken with the DNase "nicking" step to prevent excessive probe digestion. For shorter probes, Terminal Labeling and *Bio-Bridge*™ systems can be used.

Terminal Labeling Kit*

An alternative labeling technique developed by Enzo is the terminal extension of DNA probes with a polynucleotide "tail" containing biotinylated residues.

Terminal labeling is based on the use of terminal deoxynucleotide transferase (2) to catalyze the 3'-hydroxyl addition of Bio-11-dUTP, resulting in DNA probes with single-stranded biotinylated "tails". Since the 3'-hydroxyl termini function as initiation points for nucleotide addition, the number of "tails" and, therefore, the extent of biotinylation is related to the number of termini. Large DNA sequences can be cleaved (with DNase I or restriction endonucleases) to fragments 200-500 nucleotides to generate an increased number of 3'-hydroxyl termini.

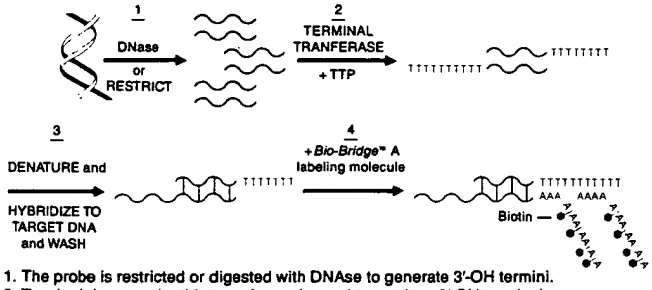
Using Enzo's Terminal Labeling Kit, double-stranded DNA with recessed, protruding or flush 3'-hydroxyl termini can be labeled. Unlike nick translation, terminal labeling is template-independent, there is net DNA synthesis and the hybridizing sequence is not modified. Also, in contrast to nick translation, single-stranded DNA, cDNA and short and long DNA fragments can be labeled.

Bio-Bridge™ Labeling System*

The *Bio-Bridge*™ labeling system is an indirect method for the delivery of biotin label to DNA probes by a polynucelotide moiety that contains biotinylated residues and is capable of hybridizing to the probe DNA.

As diagrammed below, this labeling system uses terminal deoxynucleotide transferase to add unmodified 3'-poly T tails to the DNA proble. The poly T-tailed probe is hybridized to the target DNA sequence and then to the *Bio-Bridge*™-A labeling molecule which is a biotin-modified poly dA. Because of the low complexity of the poly dA sequences of the *Bio-Bridge* molecule, hybridization to the poly T-tailed probe is virtually instantaneous.

The Bio-Bridge™ labeling system



1. The probe is restricted or digested with DNAse to generate 3'-OH termini.
2. Terminal deoxynucleotide transferase is used to catalyze 3'-OH terminal addition of a hompolymer of TTP.
3. The T-tailed probe is denatured and hybridized to target DNA.
4. *Bio-Bridge A* labeling molecule is hybridized to the poly T-tails to bridge the hybridized probe to one of the *Detek*™ signal generating systems.

This novel method of indirect biotin-labeling provides all of the advantages of terminal labeling. It can be used with small probes, single-stranded or double-stranded DNA as well as with cDNA. In addition, the *Bio-Bridge* labeling molecule can be used as a universal label for any poly T-tailed probe and therefore it is not necessary to biotinylate individual probes.

The *Bio-Bridge* procedure provides the advantage of a low background because the probe is unlabeled. Also, this method can deliver a greater number of biotin molecules to the hybridized probe, providing a better sensitivity than with probes biotinylated by nick translation or terminal labeling.

ribo *Bio-Probe*™ System*

The ribo *Bio-Probe* system is a method for the production of single-stranded, biotinylated RNA probes through transcription of cloned sequences.

The DNA sequence of interest is first cloned into the M13 polylinker region located downstream from an SP6 promot r in the plasmid vectors pSP64 or pSP65. These plasmids differ only in the orientation of the polylinker. When the DNA is cloned into any of the restriction sites in the polylinker segment, RNA transcripts from this DNA can be produced by the SP6 RNA polymerase (4).

Using biotinylated UTP (Bio-11-UTP) together with ATP, GTP and CTP, the resulting transcript is biotinylated at all U residues and is suitable for use in hybridization studies.

*Patent Pending

Bi tinylat d nucl tid s* are applicable to a wide variety of systems. We have summarized current data for th benefit of those scientists who wish to adapt their research procedures to the use of biotinylated nucleotides.

Nucel tid	at. No.	
Bio-11-dUTP Bio-16-dUTP	EBP-806 EBP-811	Both nucleotides replace TTP in nick translation. The rate of incorporation is slightly slower for the 16-atom linker nucleotide. Enzo scientists have determined that these nucleotides provide equal detection sensitivity. Biotinylated dUTP is also a substrate for T4 polymerase, α and β polymerases from murine (A-9) and human (HeLa) cells and the DNA polymerase of herpes simplex virus I (2). Biotinylated dUTP is not a suitable substrate for avian myeloblastosis virus reverse transcriptase (2).
Bio-11-dCTP	EBP-816	Biotinylated dCTP can be used in place of dCTP in a standard nick translation reaction. This nucleotide is especially suitable for nick translation of GC-rich sequences.
Bio-11-dUTP Substrate Mix Bio-16-dUTP Substrate Mix	EBP-810 EBP-812	These substrate mixtures have been optimized for terminal labeling and contain, in addition to Bio-dUTP, the unmodified nucleotides TTP and dCTP.
*Bio-Bridge™-A** Labeling Molecule	EBP-814	The *Bio-Bridge-A* labeling molecule, a biotin-modified polymer of dAMP, can be used as a indirect label for poly T-tailed DNA probes (see diagram on previous page).
Bio-11-UTP	EBP-815	The biotinylated ribonucleotide Bio-11-UTP can serve as a substrate for the SP6 RNA polymerase in the specific transcription of cloned sequences from the SP6 promoter. The nucleotide can substitute for UTP in reactions catalyzed by the RNA polymerases of *E. coli* (2,5) and bacteriophage T7 (2). The biotinylated ribonucleotide is a less efficient substrate for RNA polyerases than the biotinylated deoxyribonucleotide is for DNA polymerases. The ribonucleotide is utilized poorly by the eukaryotic RNA polymerases (HeLa cell RNA polymerase III, calf thymus RNA polymerase III and mouse L-cell RNA polymerase II) (2).

REFERENCES:

1. Rigby, P.W.J., *et al.*(1977) J. Mol. Biol. *113*, 237.
2. Langer, P.R., *et al.* (1981) Proc. Natl. Acad. Sci. USA *68*, 6633.
3. Bollum, F.J., (1974) *The Enzymes* (Boyer, P.D., ed.) *10*, 148 Academic Press, New York.
4. Green, M.R., *et al.* (1983) Cell. *32*, 681.
5. Hutchison, N., *et al.*. (1982) J. Cell. Biol. *95*, 609.

Enzo offers a variety of DETEK® Signal Generating Systems for visualization of biotinylated nucleic acid probes. To determine which DETEK system is most appropriate for your research needs, ask for a FREE copy of our USER'S GUIDE TO DETEK Signal Generating Systems and a complete catalog of Enzo products.

ORDERING INFORMATION

DNA and RNA Lab ling Kits

Kit	For Labeling	Cat. No.	Pric
Nick Translation Kit	15μg DNA	EBP-803-15	$175.00
Nick Translation Kit	8μg DNA	EBP-803-8	110.00
Terminal Labeling Kit	15μg DNA	EBP-809-15	225.00
Terminal Labeling Kit	8μg DNA	EBP-809-8	155.00
Bio-Bridge™ Labeling System	8μg DNA	EBP-813-8	155.00
Bio-Bridge™ Labeling Molecule	8μg DNA	EBP-814-8	110.00
ribo *Bio-Probe*™	10-50μg plasmid DNA	EBP-817-10	225.00
Labeling System	5-25μg plasmid DNA	EBP-817-5	120.00

Biotinylated Nucleotides

Nucleotide			
Nick Translation			
Bio-11-dUTP	15μg of DNA	EBP-806-15	$85.00
Bio-11-dUTP	8μg of DNA	EBP-806-8	50.00
Bio-16-dUTP	15μg of DNA	EBP-811-15	85.00
Bio-16-dUTP	8μg of DNA	EBP-811-8	50.00
Bio-11-dCTP	15μg of DNA	EBP-816-15	85.00
Bio-11-dCTP	8μg of DNA	EBP-816-8	50.00
Terminal Labeling:			
Bio-11-dUTP	15μg of DNA	EBP-810-15	$85.00
Bio-11-dUTP	8μg of DNA	EBP-810-8	50.00
Bio-16-dUTP	15μg of DNA	EBP-812-15	$85.00
Bio-16-dUTP	8μg of DNA	EBP-812-8	50.00
Biotinylated Ribonucleotide:			
Bio-11-UTP	10-50μg plasmid DNA	EBP-815-10	$150.00

For Research Purposes Only.

FOR TECHNICAL ASSISTANCE
OR TO PLACE AN ORDER
CONTACT OUR CUSTOMER SERVICE DEPARTMENT

CALL: (800) 221-7705
TOLL FREE-INSIDE
THE CONTINENTAL
UNITED STATES
(EXCEPT NEW YORK STATE)

(212) 741-3838 In New York State or outside Continental United States

WRITE: **ENZ BIOCHEM, INC.**
325 Hudson Street
New York, NY 10013

TELEX: ENZOBIO 424683

ENZ